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OF BLOOD FLOW MARKER DISTRIBUTION IN
NORMAL AND INFARCTED HEARTS

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TOPOGRAPHIC DISPLAY AND QUANTITATIVE ANALYSIS
OF BLOOD FLOW MARKER DISTRIBUTION IN
NORMAL AND INFARCTED HEARTS *

by

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ABSTRACT

Computer-based image analysis methods have been developed for the study of blood flow marker distribution in ischemic and non-ischemic hearts. The flow markers which have thus far been used are Thioflavin S and ^{85}Sr -microspheres. Original images produced by flow markers, were digitized and amplitude bands corresponding to intensity ranges of the indicators were created by Fourier-based filtration and amplitude mapping which together remove noise and demonstrate marker distribution topographically in all directions. Cartesian plots of flow marker values averaged across transmural lines and lines normal to them were made to show marker distribution along selected radial and circumferential directions in heart walls. Histograms of marker intensity have been obtained to describe distribution of marker within regions bounded by arbitrarily shaped contours drawn on images. The resultant data shows both heterogeneity and distinct gradients in normal hearts, at infarct margins, and within ischemic zones. The local distribution of flow implied by Thioflavin S was more inhomogeneous than that indicated by the microspheres, probably as a consequence of the Thioflavin fluorescence being a surface phenomenon. Advantages of these computer-based methods are that they provide a high resolution quantitative description of marker distribution and a means of topographically relating flow marker and structural changes all in a way which is both flexible and efficient for handling large amounts of data.

INTRODUCTION

Interest in determination of myocardial infarct size and in limitation of its extension has led to the use of diffusible and nondiffusible flow markers in animals which are sacrificed after injection of a marker for analysis of its distribution. Current analytic techniques which have shown that regional variations exist under normal and abnormal conditions determine volume radioactivity of discrete tissue samples or produce images which are usually examined visually without processing or quantitative analysis. However, cutting tissue into small samples is tedious and may lack spatial resolution while the image-based observations have been primarily qualitative. Therefore, there is a need for techniques which can provide high spatial resolution and quantitative analysis in a way which facilitates handling large amounts of data necessary to improve our understanding of myocardial blood flow under various conditions.

In our laboratory we have been exploring the possibility of obtaining an estimate of flow per unit mass from analysis of images produced by radioactive and fluorescent blood flow markers. In this report we describe a computer-based method for analyzing such images and present results which show the possibility of improving resolution beyond that practical with volumetric determinations. This analysis employs amplitude images of blood flow indicators observed in slices of heart and produces quantitative data giving a virtually continuous planar representation of blood flow.

METHOD

Our characterizations of myocardial blood flow marker distribution were carried out on normal hearts and on hearts following coronary ligation in mongrel dogs and Yorkshire pigs. The animals were anesthetized with thiamylal sodium, intubated, and anesthesia was maintained with 1.5-2.5% fluothane in oxygen. The heart was exposed through a left thoracotomy, and an 8F polyethylene tube inserted into the left atrium. The electrocardiogram and systemic arterial pressure were continuously monitored. Blood flow markers were injected either in this state or after a 12-370 minute period of left anterior descending artery occlusion. Microspheres (3M) labelled with ^{85}Sr and having a mean diameter of 9 ± 1 microns were suspended in dextran, agitated in a Vortex shaker, and then mixed ultrasonically for 10 minutes. Microscopic examination assured dispersion of clumps. A bolus of $1 \times 10^6 - 2 \times 10^7$ microspheres was injected into the left atrium of the animal and followed by a saline flush. A reference sample of arterial blood was withdrawn at constant rate over the next 3 minutes. Despite injection of such a large number of microspheres, no significant change of systemic arterial pressure or cardiac rhythm were noted. Immediately after microsphere injection, a 4% filtered solution of Thioflavin S (1 ml/kg) was given intravenously and the animal sacrificed. The hearts were removed, rinsed, frozen, and cut into slices 2 mm thick.

Photographs of the slices were taken under uniform ultraviolet light which causes the Thioflavin S to fluoresce. The slices were then pressed between two sheets of no-screen x-ray film and exposed for 7-10 days. Care was taken to maintain the same orientation of slices in the Thioflavin fluorescence photograph and autoradiograph so that the images could be readily compared. The availability of radiographs made from pressing film to both the basal and apical surfaces of the heart slices permitted averaging two images from the same slice to provide a better representation of the volume activity within the 2 mm thickness by compensating for absorption in the tissue and nonuniform sampling of radioactivity due to the spreading of emissions originating farther from the film plane. The distribution of blood flow indicators was thus represented by the optical density of the single or averaged autoradiographs and by the reflectance of the Thioflavin photos.

The images of the flow indicators were digitized using a television camera and shading corrector interfaced to a disk-based minicomputer system. The TV camera was fitted with a macro lens and mounted on a photo stand that provided transillumination for the autoradiographs and overhead illumination for the opaque photographs of Thioflavin fluorescence. The shading corrector compensated for nonuniform illumination and sensitivity of the vidicon as well as allowed expansion of the TV signal amplitude for a better match with the dynamic range of the A/D converter. This match was done using two neutral density filters selected individually to achieve a relative density difference that represented the range present in each photograph or autoradiograph. A typical optical density range employed for the autoradiographs was 1.2 while that for the photographs was 0.6.

The central 384 lines of the 525 line television raster were digitized. Each line was sampled with 8 bit resolution at a 10 MHz rate along the central 38.4 microseconds of its 51.6 microsecond length. Typically, a field of $90 \times 90 \text{ mm}^2$ was spanned by the 384×384 pixel image and a 256×256 pixel region within the field covering a $60 \times 60 \text{ mm}^2$ area was selected for processing. The result of the data acquisition process was a series of images in digital form with amplitudes proportional to the intensity of light transmitted by the autoradiographs and reflected by the Thioflavin photographs.

One sequence of data processing steps was employed to produce images for topographic display of flow marker distribution while another series of steps was employed for quantitative analysis of marker distribution. First, in each case, the images were linearly scaled to fill the entire 256 available amplitude values. This normalized minima and maxima of the two flow indicators being studied. The amplitude maps required to expand the images to the maximum dynamic range of our system were determined from histograms of amplitudes in the entire field of the original images. Since the areas of microsphere perfusion appeared dark on the autoradiographs and those areas containing Thioflavin S appeared light in the original images, amplitude mapping was also used to reverse the contrast of the microsphere images so that higher amplitude represented greater flow for both images.

For topographic display of marker distribution, the scaled images were smoothed using Fourier-based filtration to remove noise artifacts and show trends in the gradients of flow as topographic contours which facilitate the assessment of regional flow marker patterns. The

smoothing process was carried out in the frequency domain using operator-entered parameters to define a low pass filter having a Blackman window shape (1). For a 256×256 image spanning $60 \times 60 \text{ mm}^2$, the 256×256 point Fourier transform that we employed has a fundamental frequency of $1/60$ of a cycle per mm and harmonics up to 2.13 cycles per mm. A filter span of 97 points was used with the result that the highest non-zero spatial frequency was 0.80 cycles per mm. Based on the rule of thumb that the smoothing window is about equal to the reciprocal of the 3db bandwidth, our window corresponds to a smoothing span of 1.5 mm which is comparable to the slice thickness. This produced an easily interpreted image of flow marker distribution without the distraction of fluctuations arising from noise or other artifacts and without seriously blurring flow marker gradients. Flow marker trends were further emphasized by employing a display containing 8 amplitude ranges.

For quantitative analysis of the flow markers, the amplitude points in the images were scaled logarithmically. This processing was carried out because the photographic quantity proportional to fluorescence and radioactivity is optical density and optical density is proportional to the logarithm of light transmission or reflection. After logarithmic scaling of amplitude, histograms of operator-specified, arbitrarily shaped regions were obtained. From a region assumed to have normal flow, the mean amplitude and the standard deviation were found. Then, the mean was scaled to an amplitude of 140 and a linear mapping was employed to assign each grey level a bandwidth equal to a standard

deviation. The value of 140 was chosen to facilitate observation of both higher and lower levels on our display in view of its characteristics. This procedure, done for both types of markers, produced images in which amplitude is proportional to flow marker distribution with a common mean value and spreads represented with equivalent grey scales. In addition, average amplitudes were calculated across bands of tissue within the images. The amplitudes were displayed in Cartesian form for direct comparison of the flow marker levels in specific anatomic regions of interest such as the central part of the ischemic zone, the peripheral zone, and non-ischemic zone.

After the imaging processing was complete in one of our studies, a myocardial slice was cut into pieces whose volume radioactivity was determined for comparison with the results produced by computer-based analysis.

RESULTS AND DISCUSSION

Digital images of the autoradiographs and photographic prints were used as a starting point in our computer-based analysis. The availability of data in digital form facilitates the application of signal processing to improve image quality and standardize dynamic range before quantitative analysis. The resulting images display fluorescence and radioactivity on a common amplitude scale that allows the assessment of regional flow patterns and comparison of indicators (Fig. 1).

We have produced data that shows transmural flow marker variations on a log amplitude scale in normal dog heart muscle (Fig. 2). The unsmoothed data indicates that the blood flow marker is heterogeneously distributed but that there is a generally greater concentration of flow marker in the epicardial region than in the endocardial region. The smoothed data mapped into 8 amplitude bands emphasizes the epicardial-endocardial difference in amounts of flow marker and displays local variations of flow marker in each region by means of contour lines. The Thioflavin fluorescence images which are a surface representation of flow marker distribution show more detail and apparently have higher resolution than the autoradiographs which do not contain sharply delimited boundaries because radiation may come from throughout a volume of tissue and not always be normal to the film plane.

Images of blood flow indicators have also been obtained from ischemic dog hearts (Fig. 3). The ischemic region appears darker than the surrounding tissue and extends from the endocardial to the epicardial surface. Although the region of diminished blood flow marker concentration is evident in the unsmoothed data, the data which

has been smoothed and amplitude-mapped displays the flow marker gradients more clearly. As in the fluorescence images of the normal dog heart, the fluorescence images of the infarcted dog heart show more detail than the autoradiographs.

Infarcts in pig hearts have been imaged (Fig. 4) using the same fluorescent and radioactive markers with the result that the smoothed and mapped data brings out flow variations and trends from unsmoothed data in the manner already noted. The data also shows that the flow marker concentration changes more discretely at the margins of the pig heart ischemic zone than in the dog heart.

The demonstration of blood flow by two markers in a band of tissue has been compared by averaging adjacent lines of amplitude information and plotting the results in Cartesian form (Fig. 5). The averaging process further smooths the spatial fluctuations in both blood flow markers. The Cartesian plots show that both markers in this particular average form indicate about the same flow gradient at the lateral margin of the dog heart infarct and approximately the same level of flow in the central zone of the infarct as well as in the non-infarcted heart tissue.

Amplitude band averaging has also been used to characterize blood flow trends in different directions within the heart muscle and also to compare ischemia in dog and pig hearts (Fig. 6). Tangential band averages for typical dog and pig heart infarcts show similar flow marker gradients at the lateral margins of pig and dog heart infarcts while transmural band averages demonstrate a difference in flow marker gradient between these infarcts in dog and pig hearts.

We have also been able to obtain flow descriptions in terms of amplitude distributions and their statistics in arbitrarily shaped operator-selected areas (Fig. 7). This approach has the flexibility to eliminate contributions from artifacts such as epicardial fat in fluorescence images and poorly defined borders in autoradiographs. Our initial use of this technique has produced amplitude distribution in both fluorescence images and autoradiographs of normal dog hearts to quantify flow differences between the subepicardial and subendocardial zones (Fig. 8). The mean amplitudes were obtained from eight specific areas and then compared to a determination of radioactivity of corresponding tissue samples using a gamma counter. The results show reasonable agreement between the fluorescent marker, the autographic indication of flow, and the current standard, volume radioactivity.

Characterization of myocardial blood flow by both diffusible and nondiffusible markers has attracted the attention of other investigators who have employed two basic techniques. One is to cut the tissue into discrete samples and determine the radioactivity of each sample. The result is a measurement of average flow to the entire sample which frequently weighs 0.1-3.0 gm and may be a combination of normal tissue, border zone and severely ischemic tissue. Examples of this technique are found in work done by Domenech et al (2), Becker et al (3), Yipintsoi et al (4), and Marcus et al (5). Although higher resolutions could possibly be obtained by cutting tissue into smaller samples, this becomes tedious and, therefore, impractical for large scale use.

The other technique is to form an autoradiograph or fluorescence

image. These provide a continuous representation of flow marker distribution directly in a pictorial form which can be readily studied in various ways. Malsky et al (6) used densitometric scanning of autoradiographs in transverse and transmural planes in normal and ischemic myocardium. Partlow et al (7) reported the use of a commercial digital image analysis system to determine optical density of rat brain autoradiographs and found good correlation with an analog determination of optical density and with volume radioactivity. Kloner et al (8) employed Thioflavin S fluorescence to show coronary blood flow during acute myocardial ischemia. The image-based results obtained by these investigators, whom we have selected because their work is representative of many others, shows that the continuous representation of flow markers in images is capable of high resolution and offers the potential for further processing.

This report is intended to point out processing methods can be applied for both qualitative and quantitative analysis when large amounts of data must be handled. In their current form, the methods that we have defined appear ready for validation studies. However, additional work must be done to exploit the potential of computer-based processing methods more fully for a still better characterization of blood flow markers in myocardium.

CONCLUSION

We have developed computer-based methods to analyze images produced by both radioactive microsphere and fluorescent blood flow markers. The methods produce data describing blood flow marker distribution as contour maps with amplitude bands representing different degrees of marker concentration, as Cartesian plots of marker averages over various spatial regions within the heart, or as histograms of concentration in arbitrarily shaped, operator-selected areas. The methods are flexible to accomodate variations in the dynamic range of input data and to permit custom processing and also are efficient to allow a large amount of data to be handled conveniently with a high degree of spatial resolution. In addition, the basic techniques facilitate emphasis of features that are of interest as well as demonstration of details that may not otherwise be visible for analysis. Data obtained from application of our methods has shown heterogeneity of blood flow marker distribution in normal heart and flow marker gradients in ischemic heart, information which generally conforms to that obtained by others using volume radioactivity and analog densitometric measurements. Although our results indicate that both the non-diffusible radioactive microspheres and diffusible Thioflavin S indicators give macroscopically similar descriptions of blood flow, the two types of indicators produce images which have different detail. This appears to merit further investigation and repeated trials of our computer-based approach to obtain an improved understanding of myocardial blood flow.

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Figure 1. Original and digitized images of flow markers in infarcted hearts. Original Thioflavin fluorescence image (upper left) is shown across from the digitized version which has been linearly scaled to span the entire dynamic range of the digitizing system. An original autoradiograph (lower left) produced by ⁸⁵Sr labelled microspheres is also shown across from its digitized version which has been linearly scaled as well as contrast reversed so that white indicates the presence of flow marker instead of black as in the original autoradiograph. Although the images were obtained from different slices of the same dog heart, both demonstrate an ischemic region that was produced by ligating the left descending coronary artery and illustrate the capability of digital processing to improve image quality for observation or subsequent analysis employing a common amplitude scale for each marker. The microspheres and Thioflavin were injected into the heart of this dog 20 and 23 minutes respectively after ligation.

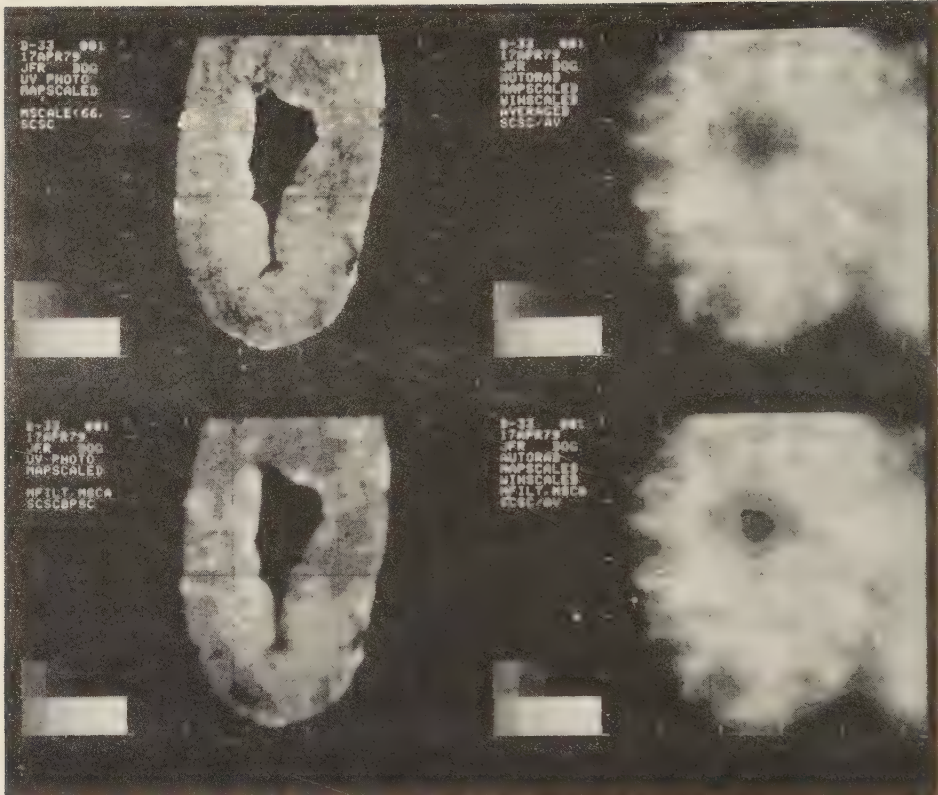


Figure 2. Flow marker images obtained from a normal dog heart. A basal surface Thioflavin fluorescence image (upper left) and a microsphere autoradiograph image (upper right) which is an average of data obtained from apical and basal surfaces of the same slice, show variations in flow marker concentration on a logarithmic scale. A smoothed version (lower left) of the Thioflavin image and a correspondingly smoothed version (lower right) of the autoradiograph image are displayed using eight grey levels to demonstrate regional flow variations by means of topographic contours which suppress point-to-point variations that obscure visual appreciation of gradients in flow marker distribution. The distribution of flow marker which is truncated at the right edge of the microsphere image is from another slice which was inadvertently placed too close on the film.

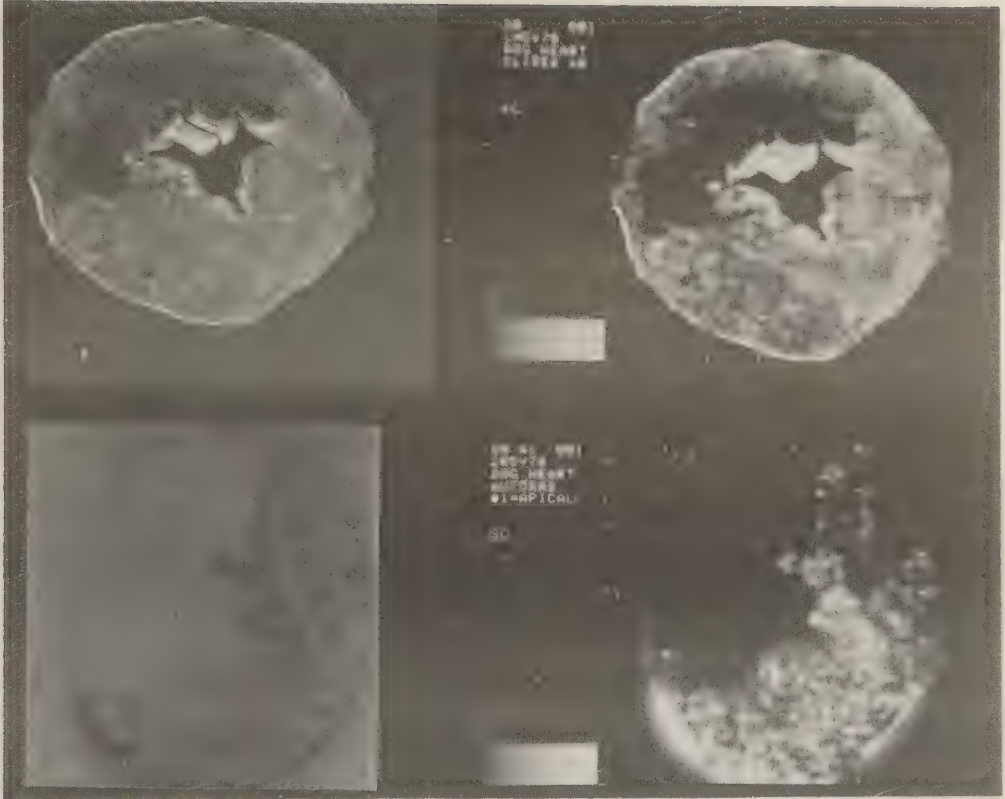


Figure 3. Flow marker images obtained from slices of ischemic dog heart. A Thioflavin fluorescence image (upper left) and microsphere autoradiograph (lower left) obtained from the same slice of ischemic heart are shown on a linear amplitude scale next to corresponding smoothed versions which have been mapped into eight amplitude bands. Although the reduction of flow marker in the area of ischemia is evident in the unsmoothed images, the flow marker gradients that exist in the tissue between the infarcted area and the normal area are more clearly demonstrated in the data which was smoothed and mapped. In this study, the microspheres Thioflavin were injected 20 and 23 minutes respectively after ligation.



Figure 4. Flow marker images obtained from slices of ischemic pig heart. A portion of left ventricular wall with a 1 hour old infarct is demonstrated on a linear amplitude scale by fluorescence of Thioflavin (upper left) and by an autoradiograph of microspheres (upper right). A smoothed version of the Thioflavin data displayed in 8 amplitude bands shows regional flow patterns (center left). A similarly processed and displayed version of the autoradiograph (center right) demonstrates flow gradients with both similarities and differences when compared to the Thioflavin image and with data obtained from the dog experiment illustrated in Fig. 3. An axonometric plot of the smoothed Thioflavin S data (lower left) and an analogous plot of microsphere data (lower right) display flow marker distribution in another form. In this slice, taken near the apex of the ischemic zone, the flow marker concentration indicates apparently normal flow in the subendocardial region with an abrupt transition to a zone of ischemia.

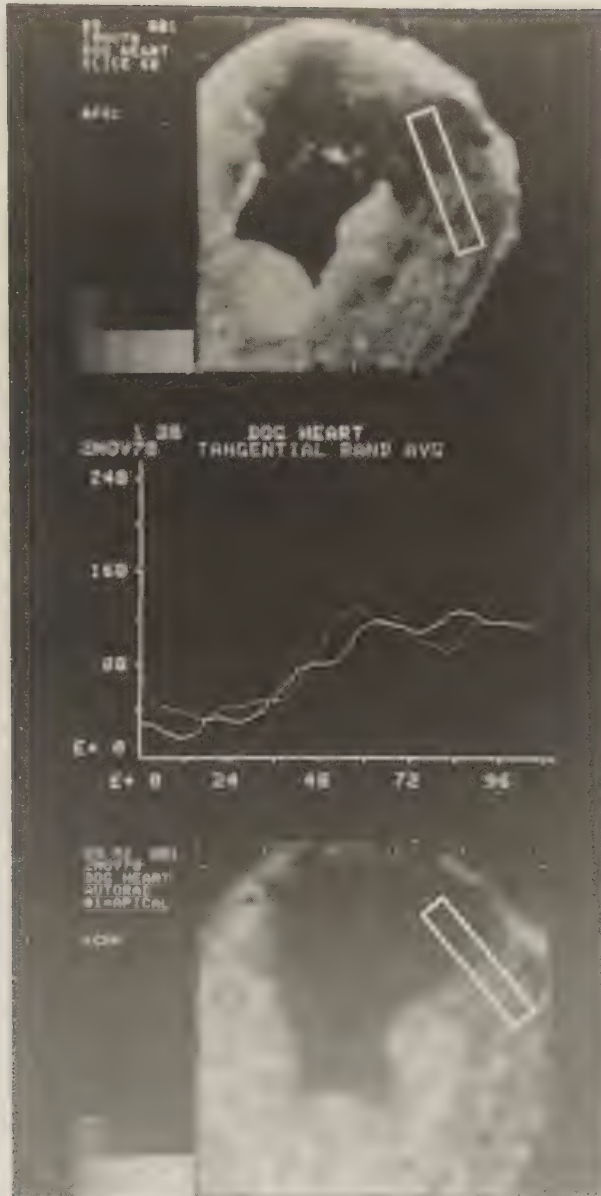


Figure 5. Comparison of flow markers in bands across a 20 minute old infarct in a dog heart. Amplitudes along the short axes of a boxed region in a Thioflavin image (upper) and in an autoradiograph (lower) have been averaged to produce the results plotted in Cartesian form (center). Similar representations of flow are given by the markers which are shown on a linear amplitude scale and have been smoothed both by an initial isotropic filtering and the subsequent transverse averaging.

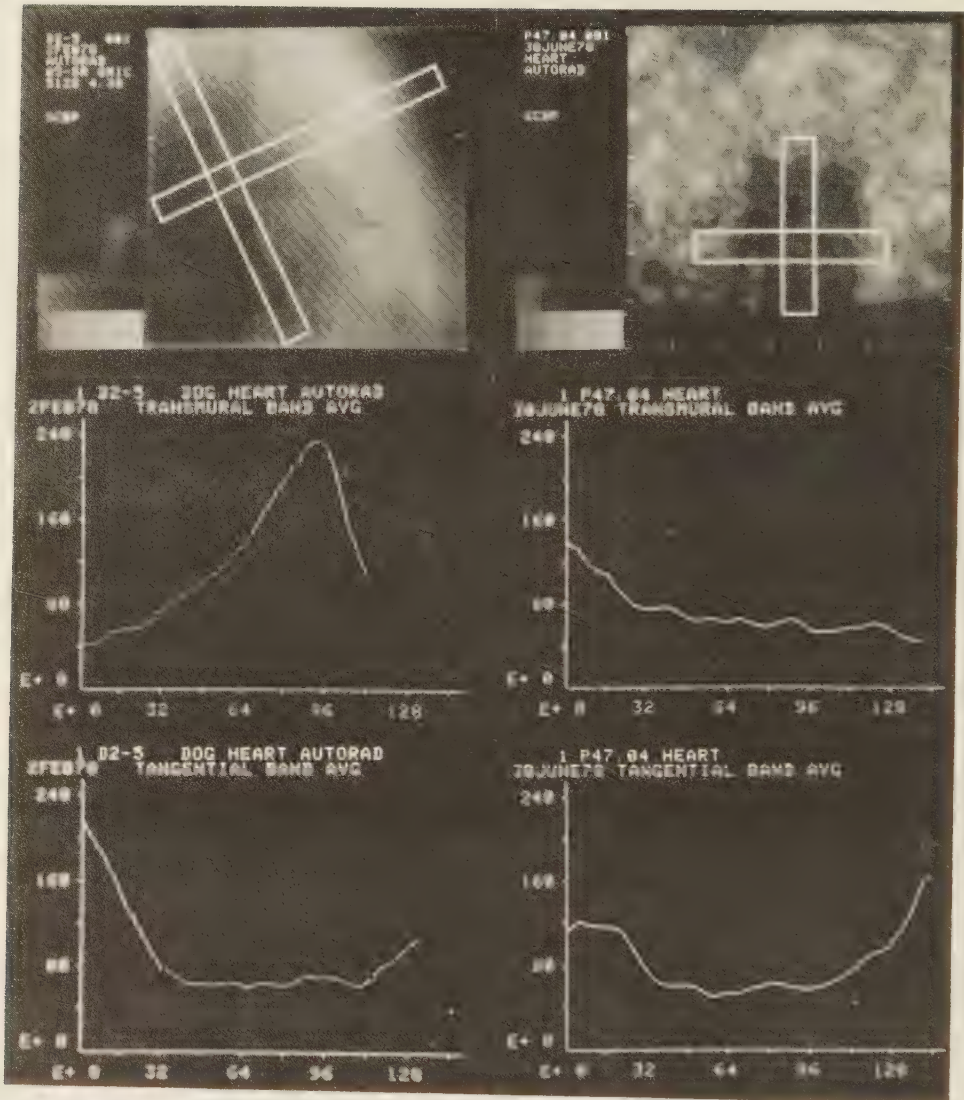


Figure 6. Transmural (endocardial-to-epicardial) and tangential band averages of flow demonstrated by autoradiography in dog and pig hearts containing 6 hour old infarcts. Amplitudes along the short axes of transmural and tangential boxes in a smoothed Thioflavin dog heart image (upper left) and in a smoothed autoradiograph (upper right) have been averaged to produce the results plotted in Cartesian form below the marker within the individual hearts as well as a difference of flow marker gradient from the epicardial surface to the endocardial surface in the two animals.

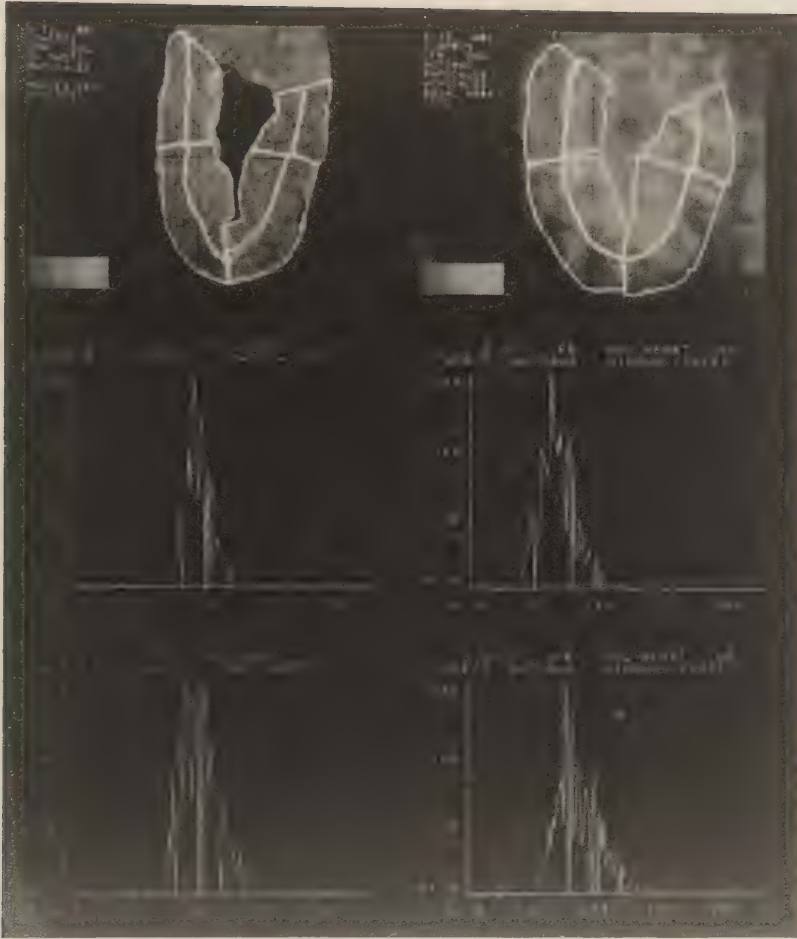


Figure 7. Flow marker histograms of subepicardial and subendocardial regions in a normal dog heart. Corresponding regions of a Thioflavin image (upper left) and an autoradiograph (upper right) are outlined for histogram analysis. The sub-epicardial region outlined in the upper left portion of each image produces a Thioflavin histogram (center left) and an autoradiograph histogram (center right) while the adjacent outlined subendocardial region yields another Thioflavin histogram (lower left) and another autoradiograph histogram (lower right). The histograms are representative of those that were also obtained for the other regions. For this analysis, the amplitudes were logarithmically scaled into a range centered at 140 with a standard deviation equal to 16.

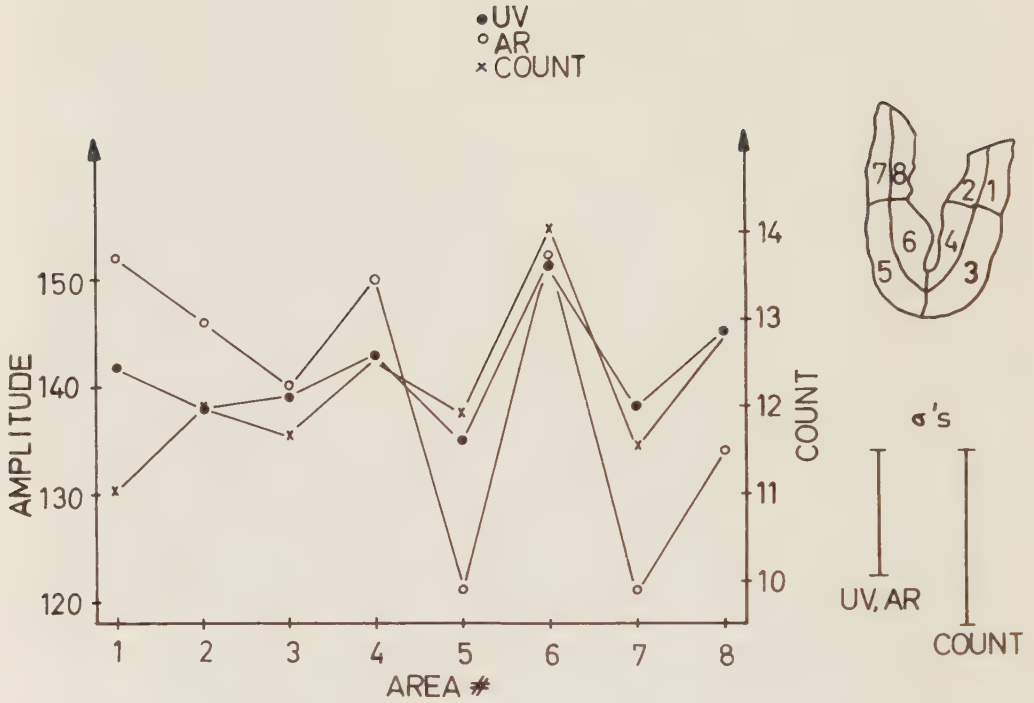


Figure 8. Regional averages of flow marker. Numbers proportional to Thioflavin fluorescence, autoradiograph density, and counted volume radioactivity are plotted for each of four epicardial regions (1, 3, 5, and 7) and four endocardial regions (2, 4, 6, and 8) outlined in the flow marker images shown in Figure 7. (AR=autoradiographic analysis, UV=Thioflavin fluorescence, Count=gamma counter determination) in (counts per minute per gram of wet tissue) 10^{-3} , σ =standard deviation)

